

CALCULATED TYROSYL CIRCULAR DICHROISM OF PROTEINS

Absence of tryptophan and cystine interferences in avian pancreatic polypeptide

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1. Introduction

Now that its crystal structure [1] has been solved at very high resolution, avian pancreatic polypeptide presents itself as a fascinating molecule in many respects. Despite its small size (36 residues) aPP exhibits characteristics pertinent to globular proteins: except for the C-terminus which is probably involved in biological activity [2], the structure is surprisingly stable. Almost all residues are organized in 2 antiparallel helices with non-polar faces the packing together of which gives the molecule a hydrophobic core. Whereas residues 14–31 form a good α -helix, proline residues at positions 2, 5 and 8 induce a left-handed polyproline II-like helix extending from position 1–8. Entropically driven formation of a very stable symmetrical dimer ($K_{12} \cong 3 \times 10^7 \text{ M}^{-1}$) [3] mainly involves the interlocking of further surface non-polar groups on the α -helices but also ionizable groups with $pK \sim 5$.

Avian pancreatic polypeptide is of special interest for the calculation of tyrosyl CD of proteins in the near ultraviolet for two reasons: (i) the high quality of the X-ray structure; and (ii) absence of tryptophan and cystine from the sequence. Due to specific difficulties encountered with the indole chromophore [4,5] studies of this kind have been restricted essen-

tially to proteins lacking tryptophan such as ribonuclease [6,7] and insulin [8–11]. The tyrosyl CD of those proteins was calculated mostly irrespective of their cystines [6,8–11]. It was thought that the underlying cystine contributions were small compared to the predominant tyrosyl CD [6,8]. Nevertheless, for a test case complete absence of cystine is to be preferred as any consideration of cystines, however sophisticated, introduces further uncertainties to the treatment.

2. Materials and methods

The CD spectra of avian pancreatic polypeptide were taken from a recent paper by Noelken, Chang and Kimmel in Biochemistry [12] with kind permission of the authors and the Journal. Calculations are based on the atomic coordinates of the X-ray structure of aPP at 1.4 Å resolution [1]. The circular dichroism of the tyrosyl L_b band was calculated as in [6] with the expression for electric dipole coupling in [14] using the electrostatic monopole/monopole approximation [13]. The far-ultraviolet CD spectrum was analyzed in terms of secondary structural composition of aPP by linear least-squares fitting with standard data in [15–17].

3. Results and discussion

In the near ultraviolet the spectrum of aPP (fig.1) differs markedly from the spectra normally encoun-

Abbreviations: aPP, avian pancreatic polypeptide; CD, circular dichroism; Tyr, tyrosine

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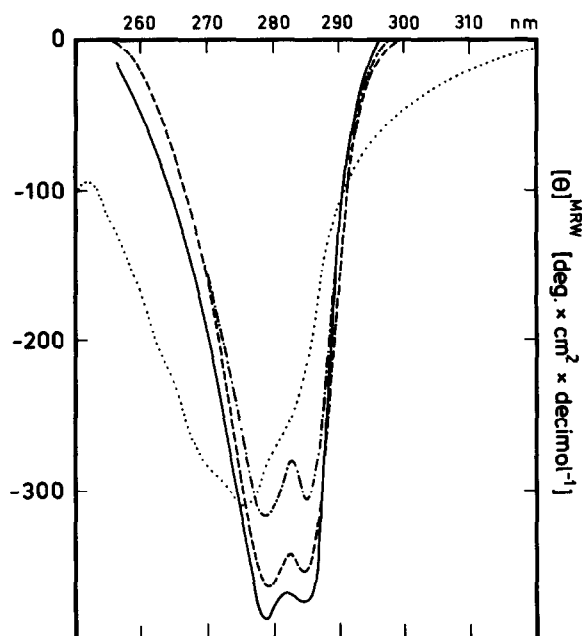


Fig.1. CD spectra of avian pancreatic polypeptide in the near ultraviolet reproduced from [12]: $c = 0.2$ mg/ml; (---) 100% dimers at pH 8 (0.1 ionic strength Tris-HCl buffer); (-.-) 79% dimers/21% monomers [3] at pH 4 (0.1 ionic strength sodium formate buffer). For comparison: (—) shape of the CD spectrum of cyclic L-Tyr-L-Tyr (inverted sign) in [19] at 297 K in ethyl ether/isopentane/ethanol (5:5:2, by vol.); (....) CD spectrum of 2 Zn-insulin at pH 7.8 (0.025 M Tris-HCl buffer); $c \sim 0.4$ mM.

tered with proteins including even those which are also lacking tryptophan or cystine. While these are broad and have a shoulder at the long wavelength side, the aPP spectrum is remarkably narrow and shows 2 nearly equivalent red-shifted peaks clearly separated by a shallow trough. This spectral phenotype strikingly resembles that of cyclic L-Tyr-L-Tyr [18,19] except for the sign. It is attributed to the special arrangement of the 2 phenolic rings folded over the same side of the diketopiperazine ring [20].

Extremely close contacts also exist in the aPP dimer between the sidechains of 2 Tyr-21. A detailed comparison of the respective geometries and a discussion of the existence of an exciton interaction in aPP will be considered elsewhere. An exciton contribution, however, would not affect the total tyrosyl rotational strength.

Results of the CD calculations are presented in tables 1 and 2. The value of $\Delta\epsilon = -4.91$ [$\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$] calculated for the electric dipole interactions

responsible for the tyrosyl CD of the aPP dimer is in good accordance with the maximum ellipticity in the spectrum $[\theta]_{\text{max}}^{\text{MRW}} = -326$ [$\text{deg} \cdot \text{cm}^{-2} \cdot \text{dmol}^{-1}$]; $\Delta\epsilon_{\text{max}} = 36$ $[\theta]_{\text{max}}^{\text{MRW}}/3300 = [\theta]_{\text{max}}^{\text{mol}}/3300 = -3.56$ [$\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$]. The agreement is still better (-3.86 vs -3.56) if one disregards Tyr-36 as a perturbing as well as a perturbed group on account of its high thermal mobility [1].

This proved a suitable measure in the case of insulin and the mobility of its A14-tyrosine and B1-phenylalanine sidechains [10,11]. Pairwise interactions contributing $>5\%$ each of the total (without Tyr-36) are listed in table 2. Roughly 50% of the CD is generated by tyrosine/tyrosine interactions. Within this class mostly Tyr-21 is involved as a perturbed group. By far the highest of all single contributions (-1.81), however, is due to the Tyr-21 pair formed upon dimerization. This contact includes the smallest monopole separation of all (1.3 \AA).

Since the L_b transition of tyrosine is polarized perpendicularly to the $C_\gamma-C_\delta$ direction of the ring it is particularly susceptible to rotation about the $C_\beta-C_\gamma$ bond. Therefore and in view of thermal mobility the effect of small rotatory deviations from the orientation defined by the atomic coordinates on the CD was calculated for the Tyr-21 couple (fig.2). For rotations of both rings about $\pm 15^\circ$ the change in total CD does not surpass $\pm 16\%$. A more detailed analysis including all aromatic sidechains as well as an assessment of the accessible angles by energy calculations will also be considered elsewhere.

A considerable increase in conformational freedom is to be expected for several sidechains upon dissociation of the dimer. Yet the values for the aPP monomer as it exists in the dimer, i.e., the half-dimer, strictly speaking, are included in table 1. Of course, the dissolution of the dominant Tyr-21 pair causes the overall CD to drop markedly. It even vanishes if the contribution of mobile Tyr-36 is again disregarded. Fig.1 includes the CD spectrum of aPP at pH 4 in a partially dissociated state. According to $K_{21} = 5 \times 10^{-6} \text{ M}$ determined in [3] the population of the solution under the conditions of fig.1 should consist of 79% dimers and 21% monomers. It can be deduced that $[\theta]_{\text{mon}}^{\text{max}} = 0.32$ $[\theta]_{\text{dim}}^{\text{max}} = -0.32 \times 326 = -104$ [$\text{deg} \cdot \text{cm}^{-2} \cdot \text{dmol}^{-1}$] and $\Delta\epsilon_{\text{mon}}^{\text{max}} = -1.14$ [$\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$], respectively. If, however, $[\theta]_{\text{mon}}$ is set to 0, the pH 4 spectrum would refer to a population of 87% dimers and 13% monomers and $K_{21} = 1.8 \times 10^{-6} \text{ M}$. It appears possible that this value is still com-

Table 1
Circular dichroism calculated for the L_b band of tyrosine in avian pancreatic peptide

Perturbing groups	Perturbed groups					
	Tyr-7	Tyr-21	Tyr-27	Σ	Tyr-36	Σ
Tyr	-0.04 <i>-0.13</i>	-0.17 <i>-2.38</i>	0.08 <i>0.08</i>	-0.13 <i>-2.43</i>	0 <i>0.02</i>	-0.13 <i>-2.41</i>
Phe	-0.25 <i>-0.57</i>	-0.09 <i>0.15</i>	0.21 <i>0.13</i>	-0.13 <i>-0.29</i>	0.01 <i>-0.02</i>	-0.14 <i>-0.31</i>
His	0 <i>0</i>	-0.02 <i>0.09</i>	0.05 <i>0.06</i>	0.03 <i>0.15</i>	-0.59 <i>-0.59</i>	-0.56 <i>-0.44</i>
Peptide bonds	0.84 <i>0.76</i>	0.38 <i>-0.28</i>	-0.96 <i>-1.20</i>	0.26 <i>-0.72</i>	-0.33 <i>-0.41</i>	-0.07 <i>-1.13</i>
Asp, Glu	0.09 <i>0.07</i>	-0.13 <i>-0.11</i>	-0.05 <i>-0.09</i>	-0.09 <i>-0.13</i>	0 <i>-0.02</i>	-0.09 <i>-0.15</i>
Asn, Gln	-0.03 <i>-0.10</i>	0.34 <i>-0.07</i>	-0.25 <i>-0.27</i>	0.06 <i>-0.44</i>	-0.02 <i>-0.03</i>	0.04 <i>-0.47</i>
Σ	0.61 <i>0.03</i>	0.31 <i>-2.60</i>	-0.92 <i>-1.29</i>	0 <i>-3.86</i>	-0.95 <i>-1.05</i>	-0.95 <i>-4.91</i>

Values are given per aPP monomer in $l \cdot mol^{-1} \cdot cm^{-1}$. Italicized figures refer to interactions within the dimer, upright figures refer to interactions within a monomer of identical geometry. The separate listing for Tyr-36 is to account for the reduced validity due to thermal mobility

Table 2
Pairwise interactions contributing >5% each to the absolute total tyrosyl CD of avian pancreatic polypeptide ($|\Delta\epsilon| = 3.82 l \cdot mol^{-1} \cdot cm^{-1}$, excluding Tyr-36)

Perturbed Tyr	Perturbing group	Smallest monopole separation (Å)	$\Delta\epsilon$ ($l \cdot mol^{-1} \cdot cm^{-1}$)
Tyr-21	Tyr-21 II	1.3	-1.81
Tyr-21	PB-25 II	2.7	-0.60
Tyr-27	PB-4	2.0	-0.59
Tyr-7	PB-8	2.1	0.45
Tyr-21	Gln-25	2.5	0.45
Tyr-21	Tyr-27 II	8.3	-0.33
Tyr-7	Phe-20 II	2.8	-0.31
Tyr-27	PB-5	3.8	-0.31
Tyr-21	PB-22	1.6	0.30
Tyr-27	Tyr-21 II	8.3	0.26
Tyr-27	PB-29	4.1	-0.26
Tyr-7	Phe-20	2.7	-0.25
Tyr-21	Phe-20 II	6.9	0.23
Tyr-7	PB-6 II	4.9	-0.23
Tyr-27	Phe-20	6.8	0.21
Tyr-27	Gln-4	3.2	-0.20
Tyr-21	PB-23	4.2	-0.20
Tyr-7	PB-11	4.8	0.20
Tyr-21	Asn-29 II	5.5	-0.19

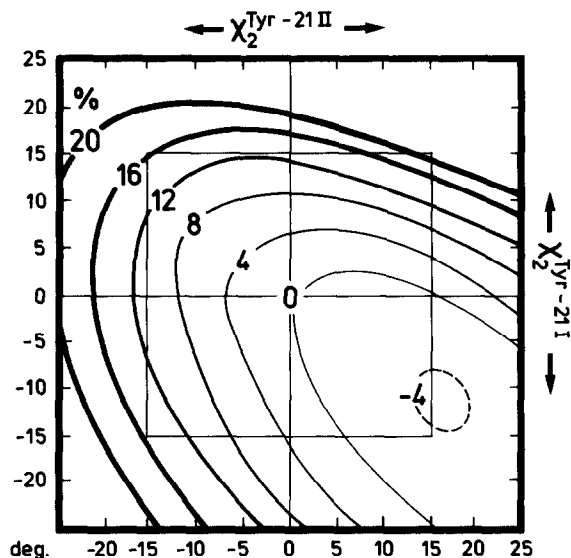


Fig.2. Effect of rotation of the Tyr-21 phenolic rings about their $C_\beta-C_\gamma$ bonds. Lines of equal deviation (4% spacing) from the CD calculated for the relative orientation defined by the atomic coordinates ($0\% \pm 0^\circ/0^\circ$).

patible with the concentration dependence of the average M_f -value, although this remains to be checked. Another argument favours a monomer CD close to 0: If, as discussed above, the 'abnormal' shape of the aPP spectrum is indeed due to the special arrangement of the Tyr-21 in the monomer/monomer interface, the dissolution of the latter should leave us with a normal spectrum, if with any. The shape of the pH 4 spectrum as compared to that at pH 8 appears essentially unchanged (fig.1). However, the fraction of monomers may be too small for a definite judgement.

Tyrosine may be involved in the generation of the circular dichroism of aPP also in the far ultraviolet (fig.3). This was considered as an explanation for the spectral differences between avian and mammalian pancreatic polypeptides [12]. These should indeed not be due to substantial differences in secondary structure since it has been shown by computer graphics that the sequence of bovine PP is well compatible with the general conformation of the avian homologue [1]. The special interaction of the corresponding tyrosines of 2 monomers, however, must be quite different because in bovine and canine PP their position is 20 instead of 21 in avian PP [21]. The far ultraviolet spectrum was analyzed in terms of secondary structural composition of aPP by computational fit with different sets of standard data [15,16]. The results

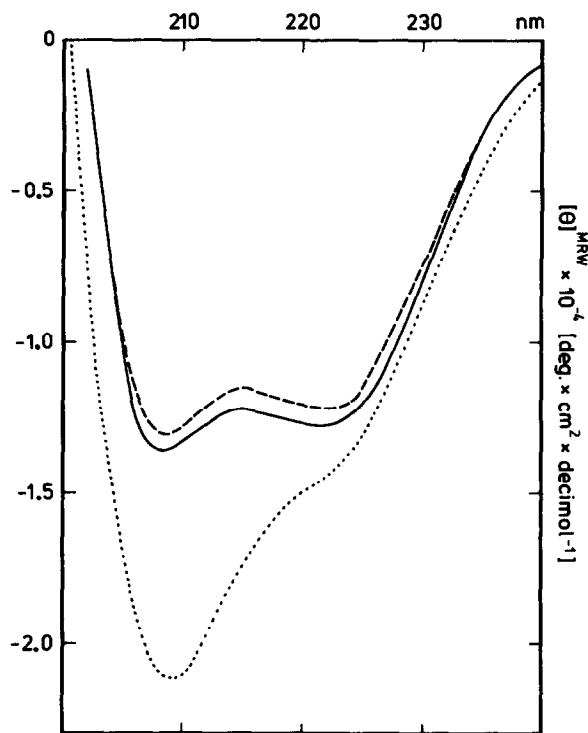


Fig.3. CD spectra of avian pancreatic polypeptide in the far ultraviolet reproduced from [12]: (—) 100% dimers; (---) 79% dimers/21% monomers [3] (conditions in fig.1); (...) spectrum simulated with the standard data from [15,17] according to the secondary structural composition of aPP identified in the X-ray structure.

are listed in table 3 together with the main-chain conformation manifest in the crystal structure. The highest fraction, though somewhat underestimated, is represented by the α -helix throughout. In one case polyproline II [17] was included in the analysis. As expected from the very high negativity of its standard spectrum, only a minute share of this conformation is compatible with the aPP spectrum. The spectral analysis further yields considerable amounts of β -structure not established in the X-ray structure and, on the whole, remains rather unsatisfactory. The spectrum corresponding to the secondary structural composition in the crystal is included for comparison in fig.3. We do not believe that the spectral discrepancies are due to substantial differences between the conformation of aPP in solution and in the crystalline state.

A spectral change accompanied partial dissociation upon lowering the pH from 8–4 (fig.3) which may reflect a conformational transition [12]. However, the more or less unaltered shape of the spectrum

Table 3
Analysis of the far ultraviolet CD spectrum of avian pancreatic polypeptide in terms of secondary structural composition

Secondary structure	Fractions (%)			X-Ray structure
	Obtained with standard data from [ref.]			
	[16]	[15]	[15,17]	
α -Helix	37.6	40.3	43.0	50
Poly(Pro II) helix	—	—	4.3	22
β -Structure	29.1	26.1	10.9	—
β -Turn	3.6	7.6	15.2	11
Random coil	29.7	26.1	26.6	17
Normalized RMS error %	4.42	8.03	5.65	—

seems to interfere with this interpretation.

A more thorough analysis comprising tyrosyl L_a -band and polyproline II contributions as well as the effect of dissociation and species differences is underway. Furthermore, the tyrosyl CD will be recalculated with a new set of coordinates which will result from the refinement of the aPP structure to 0.98 Å [22].

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